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(54) Monitoring methods and devices for use therein

(57) Methods, devices and test kits for monitoring the ovulation cycle, involve testing the body fluid, e.g. urinary, concentration of one or more analytes. Preferably estrone-3-glucuronide and luteinizing hormone are both measured, and a reference concentration for E3G is established at about day 6 of the current cycle. Preferably, disposable testing devices are used, in conjunction with a relatively permanent electronic reader/monitor. The number of "daily" tests required per month can be minimised.

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OBJECTIVES OF THE INVENTION

An objective of the present invention is to provide a system for monitoring the fertility status of an individual subject, which provides sufficient warning of the onset of the fertile phase to enable contraceptive advice to be given and which can be personalised to the individual subject, while being based solely on body fluid analyte measurements. The inherent unreliability, or limited usefulness, of other measuring systems (such as BBT) can thereby be avoided. A further objective is to avoid the use of average data obtained from population studies, with its inherent risk that in an individual subject, the parameter under test can fluctuate considerably from the population norm.

Another objective of the invention is to provide a monitoring system which is "fail-safe", in terms of advising the user of the onset of the fertile phase, without denying the user of the benefits of a simple overall procedure and limited testing regime.

A further objective is to provide the option of basing an effective monitoring system solely, or at least primarily, on the measurement of a single body fluid analyte, such as estradiol or a metabolite thereof. Other advantages of the invention will be apparent from the following description.

Another objective of the invention is to provide a testing regime which is a good balance between the desire to minimise the testing burden on the user and the need to give the user worthwhile advice about the fertility status.

Another objective of the present invention is to provide a method and devices for determining the presence and/or concentration of two or more analytes in a single sample liquid when at least one of the analytes is a multivalent analyte which is readily determined by means of two different specific binding agents in a "sandwich-format" complex, whereas another of the analytes is a monovalent analyte, such as a hapten, which is not amenable to determination via a sandwich-reaction.

It is a further objective of the invention to provide such a dual analyte assay method/device in which a particulate direct label is used to reveal the result of both assays.

The use of particulate direct labels is already known in simpler assay systems. Sometimes the use of particulate direct labels enables the assay result to be evaluated easily by eye. This may also be the case in assays in accordance with the present invention, although it is envisaged that in general the results of the assays will more conveniently and effectively be evaluated instrumentally.

A yet further object of the invention is to provide an assay method/device in which multiple analytes in a single sample liquid can be determined accurately in a strip-format assay device which is interpreted instrumentally using electromagnetic radiation (eg. light) passed through the thickness of the assay strip. The strip material can be translucent or transparent. The extent of binding of particle labels in a detection zone in the strip can provide a quantitative assay result, because the particles can block light or other radiation and therefore reduce the transmission of the radiation through the strip.

Another objective of the invention is to produce improved combinations of assay result reading devices and associated sample testing devices which can provide accurate quantitative assay information in a simple, quick and cost-effective manner.

GENERAL DESCRIPTION OF THE INVENTION

For the purposes of illustration only, the invention will be described in relation to the measurement of urinary analytes, and especially "E3G" (estrone-3-glucuronide) and "LH" (luteinizing hormone).

In addition to estrone-3-glucuronide already mentioned, estradiol metabolites that can also be assayed for the purposes of the invention include estradiol-3-glucuronide, estradiol-17-glucuronide, estriol-3-glucuronide, estriol-16-glucuronide and (principally for non-human subjects) estrone-3-sulphate. As will be appreciated from the following description, the invention can readily be applied to data derived from the measurement of body fluid concentrations of other analytes of significance in relation to the status of the ovulation cycle. Generally, the most suitable analytes are hormones and their metabolites. Follicle stimulating hormone (FSH) is an example. Examples of alternative body fluids, which are relatively accessible, are saliva, crevicular fluid, sweat, sebum, tears and vaginal fluid. In principle internal fluids, such as blood, can be used but are generally not preferred because they can only be accessed by invasive techniques.

The skilled reader will also appreciate that the body fluid "concentration" of the chosen analyte or analytes need not be measured in absolute terms, although this can of course be done if desired. Generally, it will be sufficient to assay an analyte in a manner which yields a signal, convertible to numerical data, related to the actual concentration, so that such data can be compared with similar data obtained at a different stage in the cycle to determine whether or not a significant change in actual concentration has occurred. Accordingly, where the specification and claims below refer to the "concentration" of an analyte, this expression should be interpreted broadly.

In one aspect, the invention provides a test kit for use in monitoring the ovulation cycle of a female mammal, especially a human, comprising a plurality of disposable testing devices for sampling and testing a body fluid, such as urine, and providing readable signals indicative of the concentrations of at least two analytes in the body fluid, said analytes

17. An assay device for use in the determination of two or more analytes in a single sample liquid, such as urine, at least one of said analytes being determinable by means of a sandwich-format binding reaction involving two binding reagents specific for different epitopes on said analyte and at least one other of said analytes being a hapten, and therefore not determinable readily by means of a sandwich-format binding reaction, the device comprising, preferably within a protective casing:

a) a strip (901) of porous material along which sample liquid can migrate;

b) two or more detection zones (903, 905) at least one per analyte to be determined, on said strip, located downstream from the site of sample liquid addition to said strip, of which zones:

i) at least one zone contains an immobilised capture agent being a specific binding agent for said first analyte or a specific binding agent which can capture a sandwich-format complex including said first analyte, and

ii) at least one other zone contains an immobilised capture agent which is either the hapten or an analogue thereof;

c) two or more populations of particles, located upstream from said detection zones, capable of migrating through said strip with said sample liquid, of which populations:

i) at least one population carries a binding agent specific for said first analyte, or specific for another specific binding agent also present in the device and which can participate in a sandwich-format reaction with said first analyte, and

ii) at least one other population carries a binding agent specific for said hapten;

the presence of said first analyte in said sample liquid leading to binding of particles in said at least one detection zone in an amount directly proportional to the concentration of said first analyte in said sample liquid, and the presence of said hapten in said sample liquid leading to a reduction in binding of particles of said at least one other population in said other detection zone in an amount directly proportional to the concentration of said hapten in said sample liquid, the detection zone containing the immobilised hapten or immobilised hapten analogue being preferably sited downstream from the detection zone associated with the first analyte.

18. A device according to claim 17, wherein said second detection zone is downstream from said first detection zone.

19. A device according to claim 17 or claim 18, wherein said first analyte is luteinizing hormone (LH).

20. A device according to any one of claims 17 to 19, wherein said second analyte is estradiol or a metabolite thereof, such as E3G.

21. A device according to any one of claims 17 to 20 wherein the particles are latex particles, preferably coloured.

22. A device according to any one of claims 17 to 21 wherein the affinity of the anti-hapten specific binding agent is at least about 10^9, preferably about 10^{10}, moles/litre.

23. A method of determining the presence and/or concentration of two or more analytes in a single sample liquid, at least one of said analytes being determinable by means of a sandwich-format binding reaction involving two binding reagents specific for different epitopes on said analyte and at least one other of said analytes being a hapten, and therefore not determinable readily by means of a sandwich-format binding reaction, which method comprises the steps of:

a) providing a device comprising a strip of porous material along which said sample liquid can migrate, the strip having two or more spacially distinct detection zones, at least one per analyte to be determined located downstream from the site of sample liquid addition to said strip, of which zones:

i) at least one zone contains an immobilised capture agent being a specific binding agent for said first analyte or a specific binding agent which can capture a sandwich-format complex including said first analyte,

and

ii) at least one other zone contains an immobilised capture agent which is either the hapten or an analogue thereof;

b) providing two or more populations of particles capable of migrating through said strip with said sample liquid, of which populations:

i) at least one population carries a binding agent specific for said first analyte, or specific for another specific binding agent which can participate in a sandwich-format reaction with said first analyte, and

ii) at least one other population carriers a binding agent specific for said hapten; and

c) causing said populations of particles to become suspended in said sample liquid and to migrate with said sample liquid through said strip; the presence of said first analyte in said sample liquid leading to binding of particles in said at least one detection zone in an amount directly proportional to the concentration of said first analyte in said sample liquid, and the presence of said hapten in said sample liquid leading to a reduction in binding of particles of said at least one other population in said other detection zone in an amount directly proportional to the concentration of said hapten in said sample liquid, the detection zone containing the immobilised hapten or immobilised hapten analogue being preferably sited downstream from the detection zone associated with the first analyte.

24. A method according to claim 23, wherein said second detection zone is downstream from said first detection zone.

25. A method according to claim 23 or claim 24, wherein the extent of particle binding in each of said detection zones is determined by measuring the extinction of electromagnetic radiation, such as light, when transmitted through the thickness of said strip.

26. A test kit for monitoring the fertility status of the human ovulation cycle and providing a user with an indication of the fertility status, comprising:

i) a plurality of assay devices according to any one of claims 17 to 23; and

ii) an electronic monitor comprising:

a) reading means for reading one of the assay devices;

b) information processing means for determining from said assay device reading, sample liquid concentration values for said at least two analytes;

c) information processing means and memory means for deriving from said concentration values and from previously-derived concentration values an indication of the current fertility status of a human subject under test; and

d) display means (433, 434) for communicating said current fertility status to a user of said electronic device.

27. A test kit according to claim 26, wherein said electronic device comprises receiving means (442) for receiving said assay device, said reading means being located within said receiving means, and wherein reading is achieved by optical transmission through said assay device when received by said receiving means.

28. A test kit according to claim 26 or claim 27, wherein said display means comprises one or more light sources which provide a coloured signal to the user, a variation in fertility status being indicated by a colour change.

Fig.1.

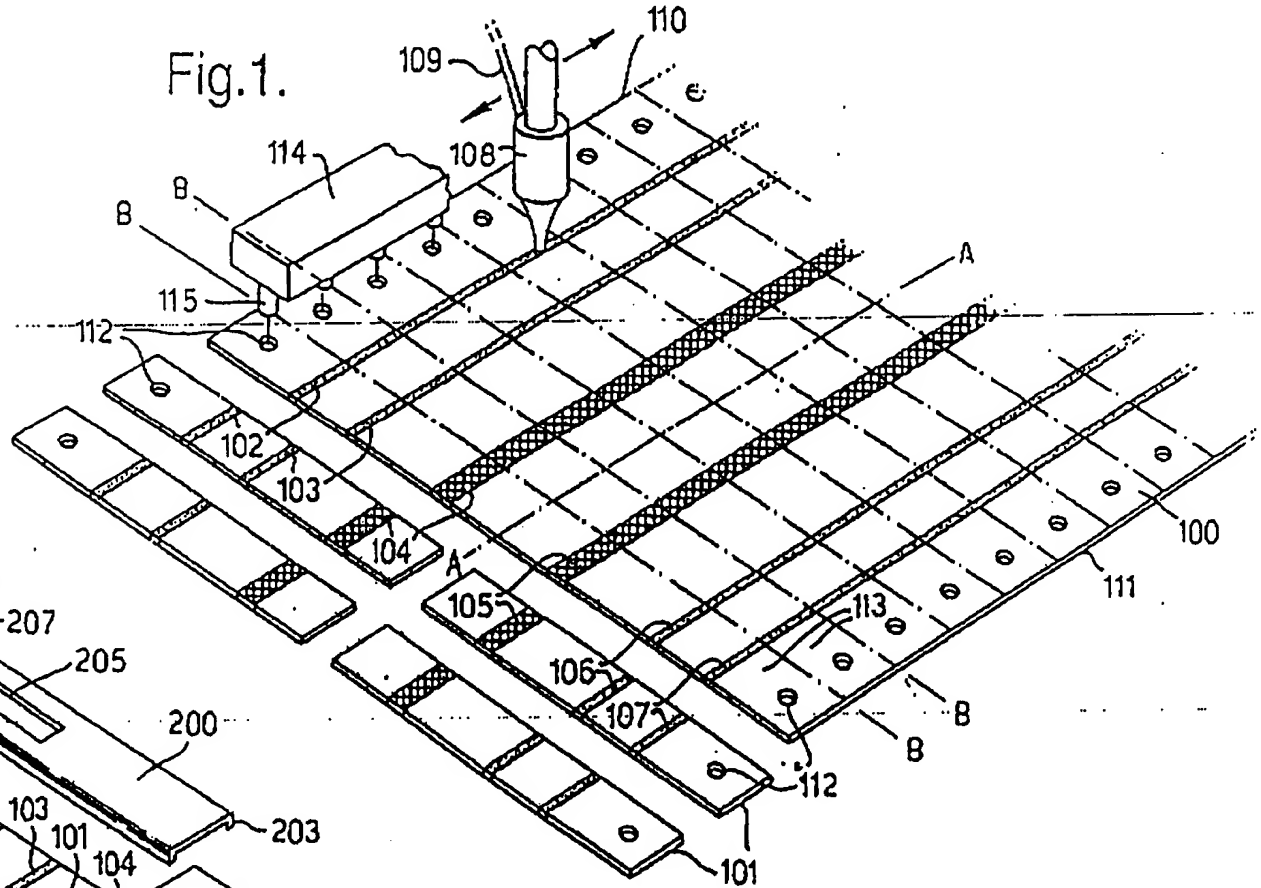

Fig.2.

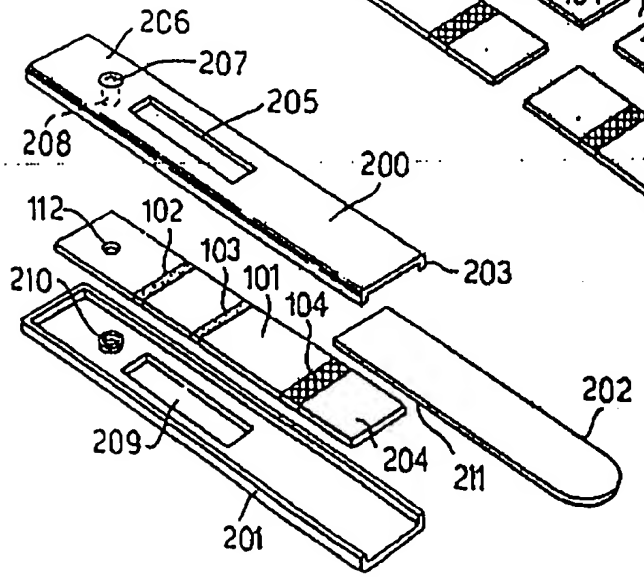

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Fig.3.

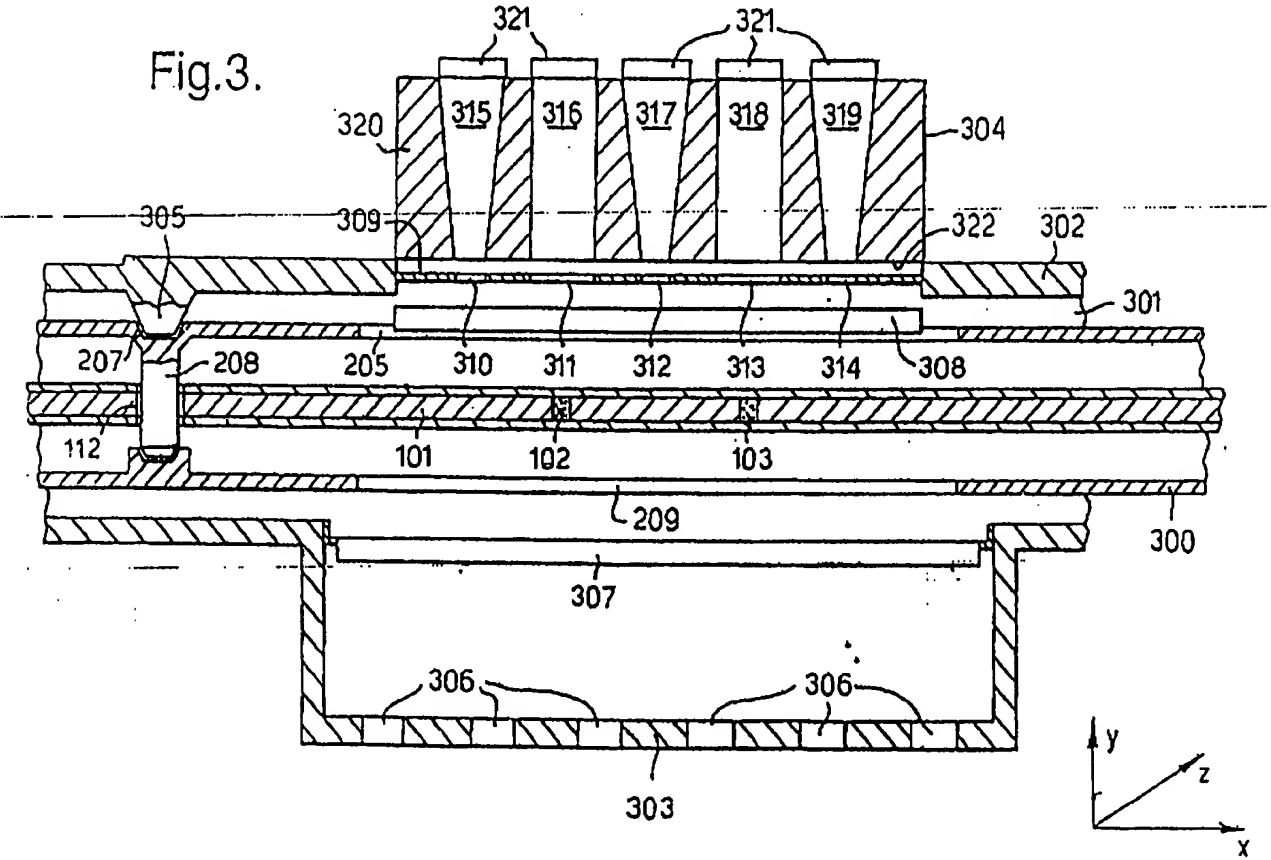

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Fig.4a.

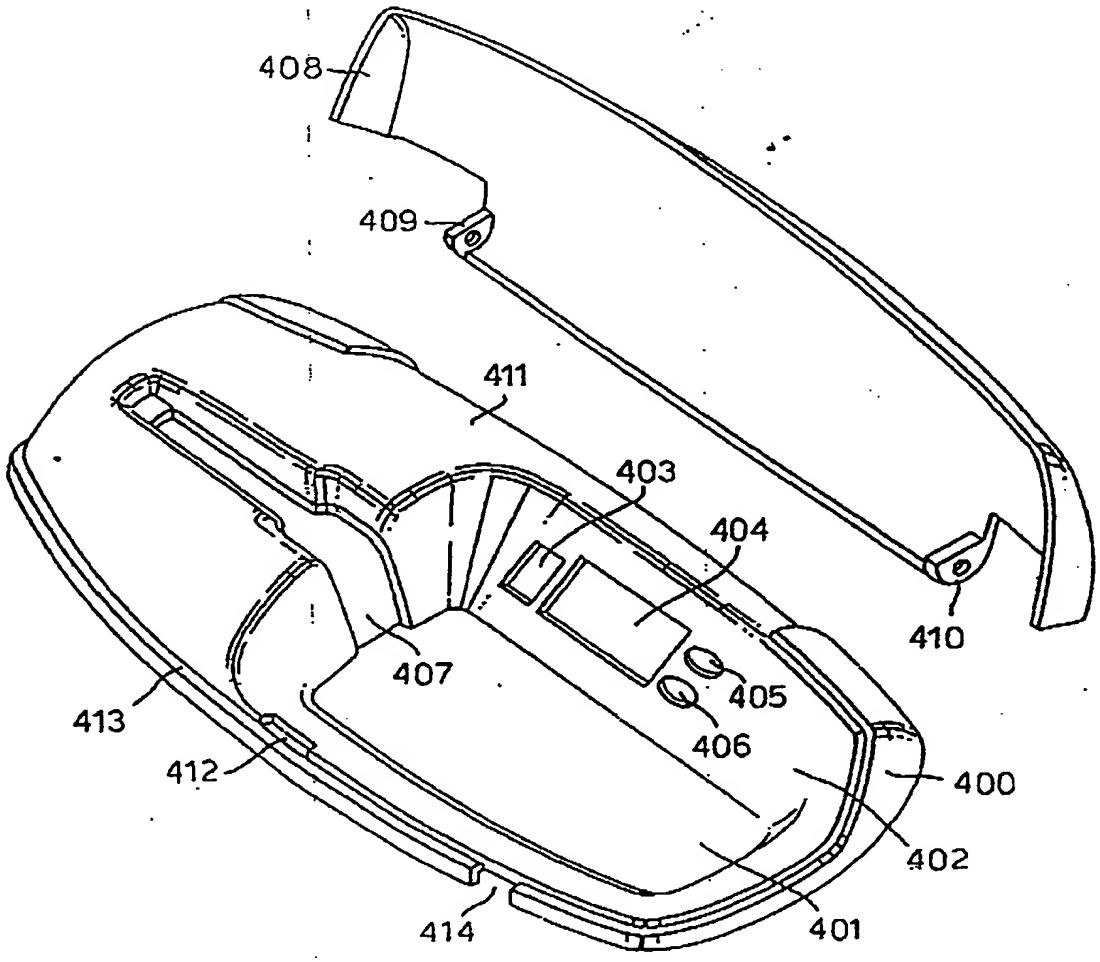
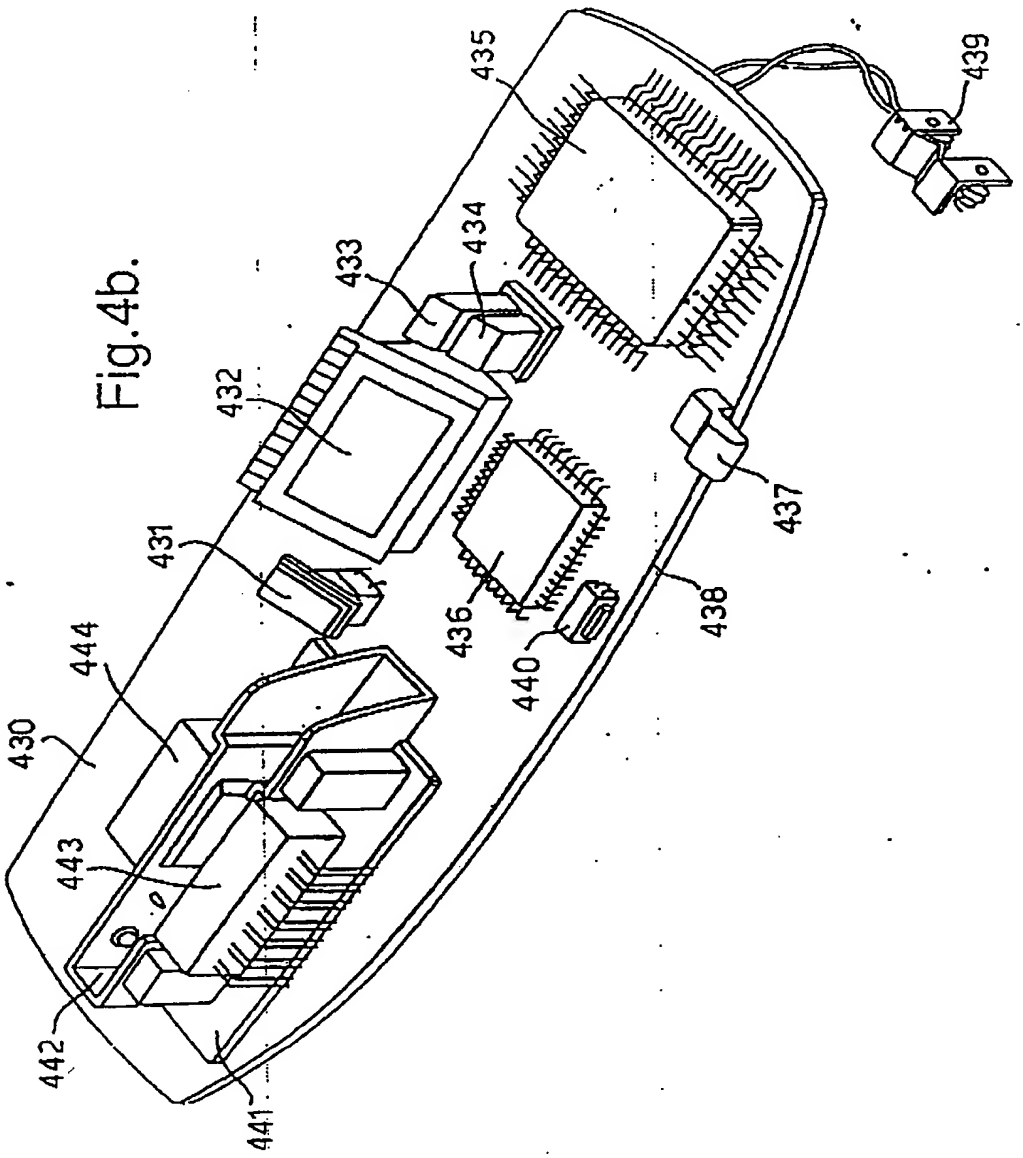


Fig.4b.



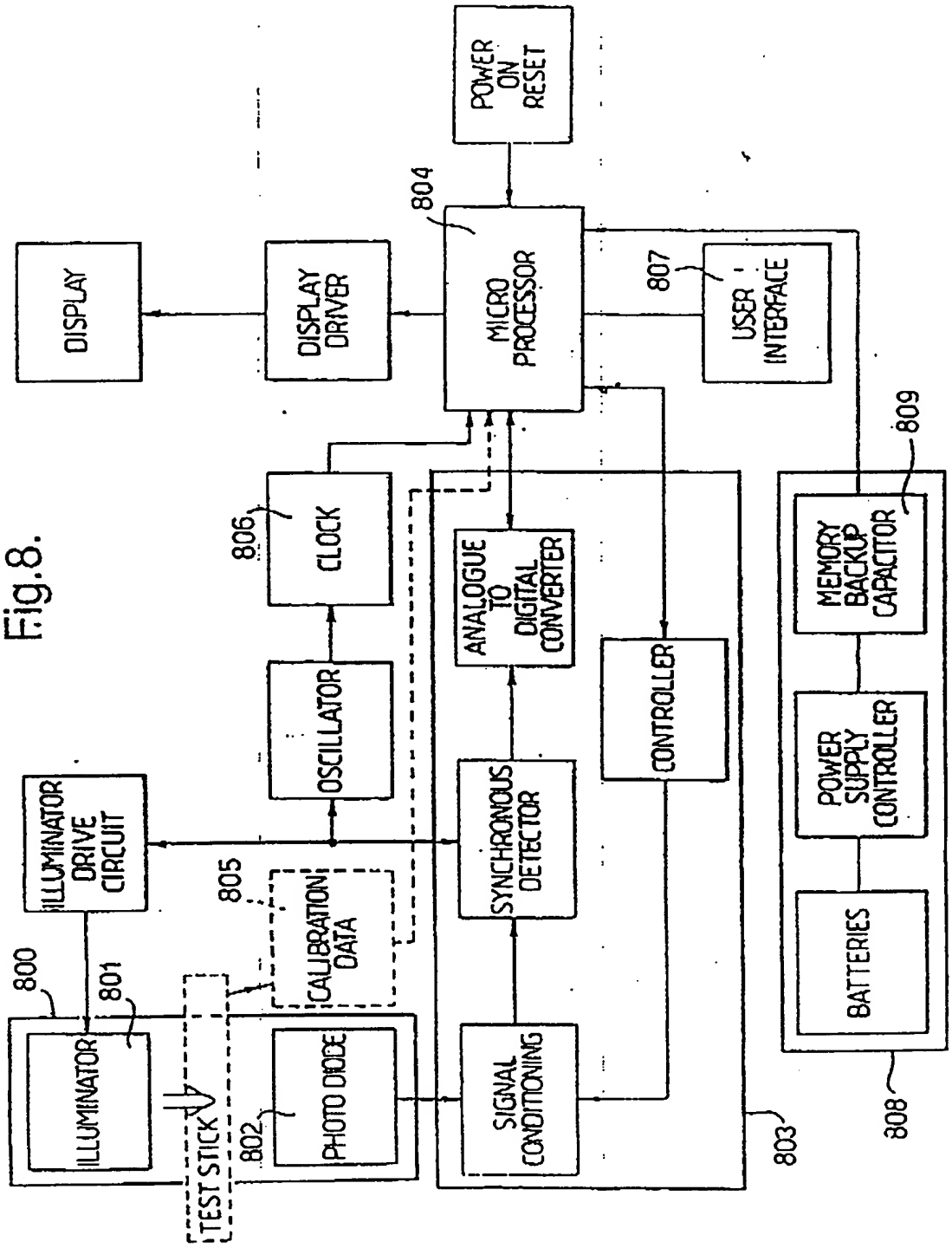

POWER ON RESET
804
DISPLAY
DISPLAY DRIVER
MICRO PROCESSOR
807
USER INTERFACE
809
806
CLOCK
ANALOGUE TO DIGITAL CONVERTER
MEMORY BACKUP CAPACITOR
OSCILLATOR
CONTROLLER
ILLUMINATOR DRIVE CIRCUIT
SYNCHRONOUS DETECTOR
POWER SUPPLY CONTROLLER
805
CALIBRATION DATA
800
801
BATTERIES
ILLUMINATOR
TEST STICK
802
PHOTO DIODE
SIGNAL CONDITIONING
803
808

Fig.8.

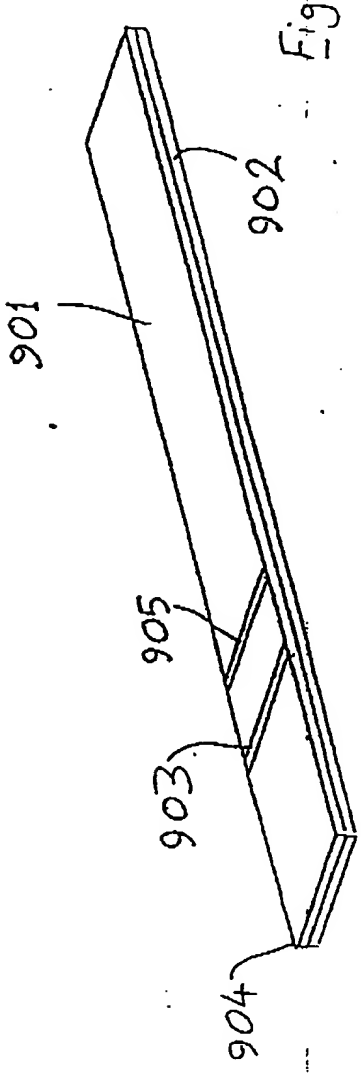


Fig. 9a

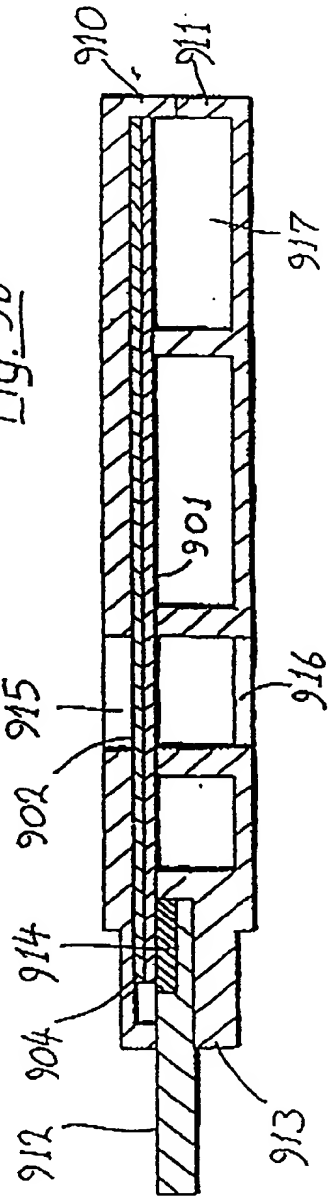


Fig. 9b

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EUROPEAN SEARCH REPORT

Application Number
EP 95 30 6661

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y A	WO-A-94 04926 (UNILEVER PLC ;UNILEVER NV (NL); UNIPATH LIMITED (GB); CATT MICHAEL) 3 March 1994 * page 8, line 4 - page 25, line 37.*	1-3,8, 17-21, 23,24,26 5,10-15, 28	G01N33/76 A61B10/00
Y A	EP-A-0 383 619 (UNILEVER PLC ;UNILEVER NV (NL)) 22 August 1990 * column 4, line 37 - column 5, line 50 * * column 9, line 2 - line 9 * * column 11, line 1 - line 9 *	1-3,8, 17-21, 23,24,26 14,15	
A	WO-A-94 02850 (MEDIX BIOTECH INC ;WELLS IAN D (US); LEIVA WILLIAM A (US)) 3 February 1994 * page 6, line 1 - page 16, line 9 *	4,25	
P,A	EP-A-0 653 625 (UNIPATH LTD) 17 May 1995 * the whole document *	1-7, 23-25	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

A61B
G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	12 January 1996	Bindon, C

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(54) Title: ASSAY DEVICES

(57) Abstract

Storage stability of an assay device, comprising an assay strip and sensitive reagents such as antibodies within a plastics casing, is maintained by moulding some or all of the casing from desiccant-containing plastics material, especially a blend of about 60-65 % polystyrene and about 30 % silica dust. Ideally the desiccant-containing plastics material is used in the moulding of a removable cap for the device. The cap can be made by sandwich injection moulding, using the desiccant-containing polystyrene as a core, surrounded by conventional polystyrene.

CLAIMS

1. An assay device comprising a casing enclosing one or more reagents which are susceptible to moisture-induced degradation during storage, wherein the casing is constructed at least in part of desiccant-containing plastics material.

2. A device according to claim 1, wherein the desiccant-containing plastics material is enrobed or shielded within non-desiccant-containing plastics material.

3. A device according to claim 1 or claim 2, wherein the desiccant-containing plastics material forms at least part of a removable cap or shroud.

4. A device according to any one of the preceding claims, comprising an assay strip or the like within a plastics casing.

5. A device according to any one of the preceding claims, wherein the portion of the device comprising the desiccant-containing plastics material is made by sandwich injection moulding.

6. An assay device comprising within a casing an assay strip together with at least one reagent in the dry state which can participate in a specific binding reaction to reveal the assay result following application of a sample liquid to the device, the casing having means whereby the sample liquid can be applied directly or indirectly to the strip, and wherein a removable cap or shroud is provided to protect the sample liquid application means, the cap or shroud incorporating desiccant in an amount sufficient to enhance storage stability of the reagent.

7. A device according to claim 6, wherein the cap or

shroud is moulded of desiccant-containing plastics material.

8. A device according to claim 7, wherein the desiccant-containing plastics material is enrobed or shielded within non-desiccant-containing plastics material.

9. A device according to claim 8, wherein the desiccant-containing plastics material comprises a blend of polystyrene and silica gel, and the enrobing plastics material comprises polystyrene.

10. A device according to claim 8 or claim 9, wherein the cap or shroud is manufactured by a process involving sandwich injection moulding.